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Baleen Whales: Preliminary Evidence for Forestomach Microbial Fermentation

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Baleen whales have a multichambered stomach divided into three distinct compartments. The forestomach (first compartment) consists of noncornified and nonglandular tissue and appears to be analogous to the tissue of the rumen. The exact function of the forestomach is unknown; however, we have detected volatile fatty acids in forestomach samples from seven bowhead (*Balaena mysticetus*) and four gray (*Escherichtius robustus*) whales. Acetic, propionic, and butyric acids were found in all samples. Seven whale samples also contained valeric, isobutyric, isovaleric, and isocaproic acids. Bacterial counts in 10 of the samples ranged from 5×10^8 to 630×10^8/ml of fluid. The types, concentrations, and relative proportions of volatile fatty acids, the presence of significant levels of bacteria, and the morphology of the digestive tract support the hypothesis that a microbial forestomach fermentation occurs in these two species of baleen whales.

Cetaceans (whales, porpoises, and dolphins) are divided into two living suborders, *Mysticeti* (baleen whales) and *Odontoceti* (toothed whales). Like ruminant mammals, baleen and toothed whales have multichambered stomachs. The three distinct compartments are the forestomach, the main or fundic stomach, and the pyloric stomach. The noncornified squamous epithelium comprising the nonglandular lining of the forestomach is analogous to the tissue of the rumen (3, 15–17). Although accurate size measurements of the mysticete forestomach have not been reported, it appears to be at least equal in size to the main stomach. This may be quite large; for example, the volume of food in the forestomach of one large fin whale (*Balaenoptera physalus*) was 550 liters (1). As in land ruminants, the size of the forestomach in fin whales is known to increase relative to that of the main stomach during maturation (17). From the forestomach, food passes to the main stomach and then to the pyloric stomach, both of which secrete digestive enzymes (16).

Although several authors have noted the anatomical and histological similarities between the cetacean forestomach and the rumen, little evidence has been reported to support the hypothesis of a microbial fermentation in this compartment. Scientists have generally considered that the forestomach of whales is adapted to take large quantities of prey on the irregular occasions prey are available (2), and prey was not believed to be digested in the forestomach (16). However, volatile fatty acids (VFAs) have been reported in the forestomachs of the small odontocetes (*Neomeris phocaenoides, Stenella attenuata, Stenella caeruleoalbea*, and *Prodelphinus (Stenella)* sp. (9–11). This finding is also consistent with a rumen-type fermentation. Since baleen whales are not held in captivity and are difficult to sample, comparable studies have not been conducted on them. This study was initiated to investigate whether VFAs and bacteria could be found in forestomach samples from two species of baleen whales, the gray (*Escherichtius robustus*) and bowhead (*Balaena mysticetus*) whales.

Forestomach contents were collected from four gray and seven bowhead whales (Table 1). Two gray whales were taken in scientific harvests during 1964 and 1968, and two had beached on separate occasions. Samples from gray whales 1968–66 and 1000 contained crustaceans. The predominant material in the forestomach of gray whale 81 was green algae (probably *Ulva* sp.). Unidentifiable crustacean fragments and algal material were found in the gray whale 78 sample. The seven bowhead specimens were killed during Eskimo subsistence hunts at Kaktovik and Gambell, Alaska. Copepods were the primary prey in three of these forestomach samples, euphausiids in two samples, and benthic amphipods in the sixth (7, 8).

Individual samples were collected by several different fisheries biologists who usually diluted and preserved the samples with buffered Formalin. Since the extent of dilution was unknown and varied among samples, a dilution factor was calculated for each based upon the chloride concentration of the sample. An undiluted and frozen sample (gray whale 78) had a salinity of 36 ‰ and served as the chloride reference sample. Chloride measurements were made with a specific ion electrode (Orion Research, Inc., Cambridge, Mass.).

Using gas-liquid chromatography methods, fluid from the samples was analyzed for VFAs including (i) the normal VFAs acetic, propionic, butyric, and valeric acids and (ii) the branched VFAs isobutyric, isovaleric, and isocaproic acids. Forestomach content samples were acidified with 50% (vol/vol) H_2SO_4. A 1-μl sample was injected into a stainless steel column (6 ft by 0.25 in.; 183 by 0.64 cm) containing 10% SP-1000–1% H_3PO_4 on 100/120 Chromosorb W AW. The column temperature was set at 145°C, and the flow rate was 120 ml of N_2 per min. The detector was a flame ionization type at 300°C (injector temperature, 160°C). Samples were analyzed on a Hewlett-Packard (5830A) gas-liquid-chromatograph with a Hewlett-Packard terminal (18850A). Lactic and succinic acids were quantified by the above chromatography procedures after methylation of the sample (4). For enumeration of bacteria, samples were diluted, passed

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TABLE 1. Characteristics and source of gray and bowhead whale specimens

Whale species	Specimen no.	Sex and size (m)	Location	Date	Food items	Time of sampling after death (h)
Gray	1000	Male, 12.7	Richmond, Calif.	3/20/64	Crab larvae	8–24
	1968–66	Female, 8.6	Richmond, Calif.	4/11/68	Crab larvae	8–24
	78	Male, 8.4	Neah Bay, Wash.	9/15/78	Unknown	48
	81	Male, 8.1	Wauna, Wash.	6/23/81	Algae	24
Bowhead	79-KK-1	Female, 12.7	Kaktovik, Alaska	9/22/79	Copepods	72
	79-KK-2	Female, 10.7	Kaktovik, Alaska	10/6/79	Copepods	18
	79-KK-3	Male, 10.3	Kaktovik, Alaska	10/8/79	Euphausiids	6–12
	79-KK-4	Male, 10.8	Kaktovik, Alaska	10/10/79	Copepods	6–12
	79-KK-5	Male, 10.8	Kaktovik, Alaska	10/11/79	Euphausiids	36
	82-G-2	Female, 8.8	Gambell, Alaska	5/1/82	Amphipods	3–7
	82-KK-1	Male, 16.0	Kaktovik, Alaska	9/23/82	ND[a]	ND

[a] ND, Not determined.

TABLE 2. Total bacterial counts of gray and bowhead whale forestomach samples[a]

Whale species	Specimen no.	Bacterial count per ml ($\times 10^8$)
Gray	1000	5.3
	1968–66	5.3
	78	7.7
	81	<1.0
Bowhead	79-KK-1	630
	79-KK-2	12.6
	79-KK-3	16.0
	79-KK-4	20.7
	79-KK-5	588
	82-G-2	48.6
	82-KK-1	5.6

[a] Counts are estimated because samples were originally diluted an unknown amount.

through Nuclepore 0.2-μm membrane filters, stained with acridine orange, and counted with an epifluorescence microscope.

Using the acridine orange microscopic enumeration technique, populations of bacteria in 10 of the 11 samples were found to range from 5×10^8 to 630×10^8/ml of forestomach fluid (Table 2). Rod-shaped bacteria predominated in the samples, although many large coccoid forms were also seen. The bacteria appeared to be heavily colonized on particulate matter and could be seen on the surfaces of zooplankton fragments.

VFAs appear to be produced by a microbial fermentation of the populations enumerated above. The types of VFAs and their relative proportions and concentrations (Table 3) were similar to those reported for cattle and sheep rumens (5). As with ruminant animals, acetic acid was the predominant VFA detected. Although propionic acid is usually the second most abundant VFA in cattle and sheep rumens, butyric acid was found in higher molar percentages than propionic acid for 9 of the 11 whales sampled. Branched-chain VFAs were in much lower concentrations than acetic, propionic, and butyric acids in the whale samples, a finding which is again consistent with rumen fermentations. Branched-chain VFAs are presumably derived from protein

TABLE 3. Normal VFAs, branched VFAs, lactic acid, and succinic acid composition of bowhead and gray whale forestomach samples[a]

Whale species	Specimen no.	Normal VFA (mol%)				Branched VFA (mol%)			Total VFAs (mM)	Lactic acid (mM)	Succinic acid (mM)
		Acetic acid	Propionic acid	Butyric acid	Valeric acid	Isobutyric acid	Isovaleric acid	Isocaproic acid			
Gray	1000	60	14	16	—[b]	3	5	1	26	—	—
	1968–66	59	14	16	<1	4	5	2	558	0.19	—
	78	48	18	20	<1	4	8	1	170	—	—
	81	78	7	15	—	—	—	—	78	—	—
Bowhead	79-KK-1	92	2	5	—	—	—	—	1,060	27.0	9.0
	79-KK-2	58	9	21	1	5	4	1	108	0.54	2.09
	79-KK-3	63	7	19	—	4	6	1	88	1.10	12.39
	79-KK-4	60	8	18	<1	4	8	2	290	—	0.09
	79-KK-5	51	16	13	3	7	10	<1	419	—	—
	82-G-2	56	9	11	4	6	6	5	136	—	tr
	82-KK-1	46	22	20	1	4	4	2	8	3.74	1.18
Gray whales											
Avg		61	13	16	<1	3	5	1	208		
Range		48–78	7–18	15–20	0–1	0–4	0–8	0–2	78–558		
Bowhead whales											
Avg		61	10	15	1	4	5	1	301		
Range		46–92	2–22	5–21	0–4	4–7	4–10	0–5	8–1,060		

[a] Total VFA, lactic acid, and succinic acid concentrations are estimated values because samples were originally diluted on unknown amount.

[b] —, Not detected.

TABLE 4. Comparable VFA and bacterial count data for forestomach regions in baleen whales and ruminants

Animal species	VFA (mol%)[a]			Total VFAs (mM)[a]	Bacterial count per ml ($\times 10^9$)[a]
	Acetic acid	Propionic acid	Butyric acid		
Gray whales	61 (48–78)	13 (7–18)	16 (15–20)	208 (26–558)	0.61 (0.53–0.77)
Bowhead whales	61 (46–92)	10 (2–22)	15 (5–21)	301 (8–1,060)	19 (0.56–63)
Sheep[b]	62 (38–69)	20 (15–30)	17 (8–22)	104 (29–187)	41 (12–88)
Cattle, cows[b]	60 (45–80)	21 (9–35)	18 (7–30)	111 (51–224)	21 (7–24)

[a] Values shown are averages. Range is shown in parentheses.
[b] Data from Hungate (5).

hydrolysis and subsequent fermentation of the branched amino acids (5).

Lactic and succinic acids were not detected in all of the forestomach samples (Table 3). With the exception of the bowhead whale 82-KK-1 sample, lactic and succinic acids were found in much lower concentrations than acetic acid in forestomach samples containing these acids.

The results of this investigation suggest that a forestomach microbial fermentation occurs in baleen whales, as evidenced by the VFAs and bacterial numbers found in the bowhead and gray whale samples. Comparable data for ruminants is presented in Table 4. As with ruminants, the concentration of VFAs and bacteria would be expected to be a function of numerous factors such as the time of sampling after the last feeding, the composition of the food items, and the age and species of the animal (5). Final validation of the fermentation hypothesis requires kinetic analysis of fermentation products from freshly collected forestomach contents and the enumeration and isolation of indigenous microorganisms capable of carrying out these fermentative activities. Although some of the samples were collected within 3 to 12 h of death, others were not as fresh, and it is possible that a postmortem fermentation occurred.

It will be of interest to determine what substrate(s) is being used in the fermentation and the actual contribution of the forestomach microbial population to the nutrition of baleen whales. Land ruminants feed largely upon plant material containing starch, cellulose, and other polysaccharides. In contrast, baleen whales feed primarily on chitinous invertebrates and small fish (6, 12, 13). Bowhead whales consume pelagic crustaceans, including euphausiids and copepods (6), whereas the major food items of gray whales are benthic fauna, mainly amphipods (18). In addition, kelp has been found in the forestomachs of gray whales, especially while these whales reside in their wintering areas (M. K. Nerini, *in* M. L. Jones, S. Swartz, and S. Leatherwood (ed.), *The gray whale* [in press]). The biochemical composition of planktonic and epibenthic prey items consumed by bowhead and gray whales is: protein, 32 to 59% (dry weight); lipid, 15 to 52%; carbohydrate, <1 to 4% (14). Is it possible that chitin, a polymer of *N*-acetylglucosamine, invertebrate protein, or lipid material is serving as the substrate for a fermentation process?

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